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Effect of water and tri(ethylene) glycol on the rheological properties of zein ☆

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Abstract

Before conducting larger scale studies utilizing highly concentrated zein blends on an extruder, it is advisable to carry out experiments on a torque rheometer. Water and tri(ethylene glycol) (TEG) are known plasticizers for zein, however, the effects these materials have on zein rheology has not been studied using a torque rheometer. The amount of each plasticizer in zein was varied between 5 and 30%. It was demonstrated that water and TEG interact differently with zein. When the amount of water was above 10% at 90 °C, torque increased rapidly. With TEG, torque increased linearly with time and only at 120 °C did the rapid torque increase occur. Multiple torque increases for zein mixtures were observed, suggesting that processes that increase viscosity are an on-going process. Significant cross-linking is not the main source for the increased torque. The energy of activation of flow was determined when using 20% TEG. A model was developed relating %water, %TEG, temperature and rpm with initial torque.

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Keywords: Zein; Plasticizer; Rheometry

1. Introduction

New industrial markets need to be found for the corn protein, which is predominantly zein, produced from the corn refining and bioethanol industries. The most successful commercial application of zein was in the fiber market where it was used as an alternative to wool (Vicara™ [1]) [1,2]. Zein fibers originally had deficient physical properties when compared to wool; these deficiencies were mitigated by treating the fiber with formaldehyde [3–7]. With the growth of synthetic alternatives (such as those based on polyacrylonitrile), the Vicara™ business eventually disappeared. The global market for wool and acrylic fiber is approximately 1.3 and 2.7 million metric tons per year, respectively [8]. A 1% penetration into these markets would allow the use of approximately 100 million pounds of zein. Efforts are currently underway to reduce the cost for isolating the zein to allow it to be competitive with petroleum based products [9]. A new high value end use for a by-product of the bioethanol industry that is renewable and biodegradable would greatly improve the economics of this industry. Given that large volumes of formaldehyde require significant investment to handle it safely, other methods must be evaluated to improve the physical properties of zein and reduce the impact of moisture so that zein can again be used in the fiber sector. Zein fiber has been produced via wet spinning methods without the use of formaldehyde, however, large amounts of other additives or solvents were needed and the fibers produced were still greatly impacted by humidity [10,11].

Melt spinning provides many commercial advantages relative to wet spinning; however, melt extrusion of zein has had mixed results [12,13]. With the flexibility that extrusion brings to many processes, obtaining additional fundamental information regarding the rheology of zein blends in the

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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melt, would increase the chance of developing an extrusion based fiber process. The rheology of zein in aqueous ethanol solutions has been studied [14–16] as well as zein with moisture contents of 15 to 35% at low shear rates [17]; however, information at higher solids and shear rate would be valuable before extensive extruder studies are begun [18–22].

2. Experimental

2.1. Materials and equipment

Zein grade F4000 obtained from Freeman Industries LLC, Tuckahoe, New York and was used as received (Lot F40000271C, 90.07% protein, 1.9% fat, 0.11% fiber and 1.53% ash). Moisture content of the supplied zein was measured at various times during testing and would vary between 3.4 and 3.6%. Deionized water was used to adjust moistures to the desired level. Tri(ethylene glycol) (TEG) was used as received from Aldrich Chemical Company, Milwaukee, WI. Glacial acetic acid was used as received from EM Science, Gibbstown, NJ. A Haake Fisons, Rheocord 90, using the 600 mixing bowl (Thermo Electron Corporation, Madison, WI), torque rheometer was used to make the torque measurements on the blends. Torque curves presented have been smoothed using one and one half minute moving averages. Individual torque values presented are one and one half minute averages. High shear roller rotors were used. Initial temperature of the chamber walls of the torque rheometer were varied between 75 and 120 °C. The temperature of the blend while being sheared was not controlled. The rpm of the rotors of the rheometer was varied between 35 and 65 rpm.

2.2. Blend preparation and mixing

To the designated amount of zein was added the reagents and the blend was initially stirred with a spatula to provide a crude blend. For the runs where only 45 g of zein (dry basis) were used, the blend could be added to the Haake rheometer in approximately 1 min. This did not completely fill the chamber and at higher run times, the torque values became somewhat noisy due to the voids that were generated during the run. When 60 g of zein (dry basis) was used, the chamber was filled near capacity; however, it typically took approximately 4 min to fill the chamber. If the chamber is filled too quickly when 60 g of zein is being tested, packing will result and hinder complete addition of material. The start time ($T = 0$) for all tests is 1 min after complete addition.

Water was evaluated as a plasticizer in the range of 5–30% (dry basis). TEG was evaluated as a plasticizer in the range of 10–30% (dry basis). TEG and water combinations were also tested where the TEG was varied from 10 to 17% and water was varied from 2.3 to 20% (dry basis). Detailed in Table 1 is a sample composition where 10% water and 17% TEG were added to the zein.

3. Results and discussion

3.1. Water series

Zein requires a plasticizer to lower the Tg of the blend to the extent that degradation will not occur during processing to allow dough formation to take place [23]. It has been known for sometime that water is an excellent plasticizer for zein [17,23,24]. When 5% water is added to the zein, the material's initial torque can be measured and the blend can be processed for a short time at 90 °C and 50 rpm (Table 2). Shortly after addition, the temperature and torque of the blend increases quickly, to 168 °C and 56 Nm, respectively, and the test was terminated. When the amount of added water was increased to 10%, the initial torque is further decreased and the blend can be processed readily at these same conditions and torque data was collected for an entire run. When the amount of added water was increased to 12.5, 15 or 20% at these same conditions, the initial torque was further reduced; however, shortly after addition there appeared a very rapid rise in torque (Fig. 1). As can be seen at all time intervals, the torque as measured is reduced with higher amounts of water, which is expected for a plasticizer. Detailed in Fig. 2 is the initial (one minute into the run), torque values versus percent added water. Over the range of values tested, the torque decreases exponentially with added water. This exponential relationship between measured torque and %water is also observed 5, 10 and 20 min into the experiment as well. When the amount of water is 15% or higher, a second rapid rise in torque is observed. As the amount of water is increased, the time at which this second rise takes place decreases. At 30% moisture there is observed a third smaller torque rise occurring fifteen minutes into the experiment.

The percent increase in torque 10 min into the experiment is exponential with increasing amounts of water (Fig. 3). This same relationship is observed at other times as well. As previously described, when the amount of water is increased, initial torque decreases exponentially. Exponential changes in torque that occurs during a run or as water is added or removed may make developing a commercial process more difficult.

When the amount of water is increased beyond 10%, in

Table 1
Example composition, 10% water and 17% TEG where zein had 3.5% moisture

Grams zein dry basis	Grams zein[a]	Grams water	Grams TEG
60.0	62.2	6.0	10.2

[a] Amount of zein actually used, equaling 60 g zein dry basis divided by 0.965 to account for water present.

Table 2
Impact of water and TEG on torque

% TEG	% Water	Torque (Nm)			
		1 min	5 min	10 min	20 min
0	5	27.1	30.5	47.5	[a]
0	10	9.1	12.9	16.2	20.2
0	12.5	5.5	9.9	11.8	14.7
0	15	3.4	8.7	9.0	13.2
0	20	1.5	8.1	8.2	12.0
0	30	0.4	1.8	4.3	5.7
10	0	38.6	42.0	43.3	[a]
15	0	28.1	31.9	34.8	39.1
17	0	26.5	29.1	31.3	33.2
20	0	17.4	19.3	21.1	24.2
30	0	5.8	5.8	6.0	6.5
10	3.9	20.1	22.7	27.3	[b]
17	3.3	12.3	15.2	17.8	21.2
17	3.9	7.4	13.1	15.7	19.3
17	4.4	7.9	11.8	14.9	18.1
17	5.5	5.9	11.3	13.6	16.2
17	7	3.5	9.7	13.9	16.5
17	10	2.1	10.0	12.5	14.3
17	15	1.2	8.4	11.3	17.2
17	20	0.6	5.5	10.4	14.7

To the appropriate amount of zein (dry basis) is added the indicated amount of either TEG and/or water. The blend is then mixed in the Haake rheometer and the torque measured at the specified time.
[a] Test stopped prematurely due to excessive temperature rise.
[b] Test stopped prematurely as rapid rise had not occurred.

spite of the lower initial torque, the higher amount of water leads to a rapid increase in torque early into the run (*vide supra*). This phenomenon has been observed by workers studying corn gluten meal under different processing conditions and deserves additional discussion [25].

Because the torque rheometer is run at atmospheric pressure, water vapor can escape during an experiment. It is

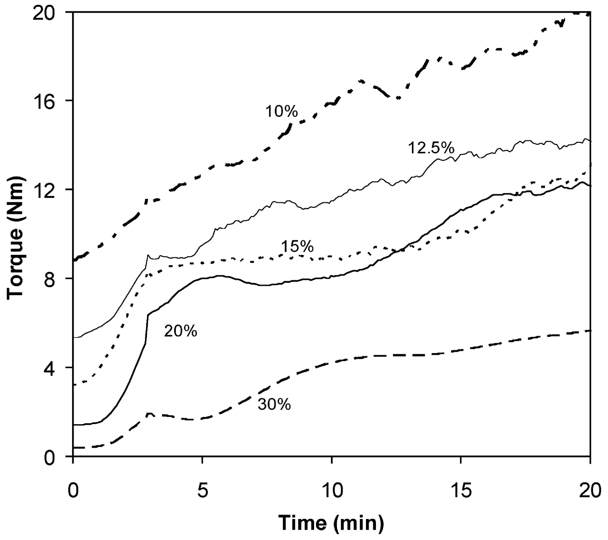

Fig. 1. Torque versus time for zein with various amounts of water. Amount of water is expressed in % of zein (dry basis) and is detailed next to the respective trace.

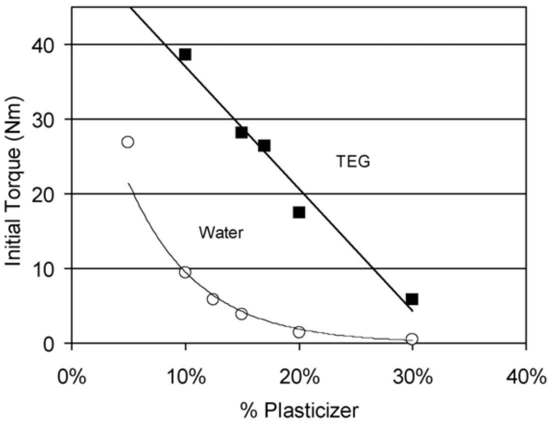

Fig. 2. Initial torque versus % added plasticizer 1 min into run. Equation for linear line fitted to TEG data, $y = -163.62x + 53.372$ $R^2 = 0.973$. Equation for exponential line fitted to water data, $y = 48.323\ e^{-16.249}$ $R^2 = 0.982$.

possible that the increased torque may be a direct result of water leaving the sample during the run. The initial torque for zein having 12.5% added water is approximately the same as that of the 20% added water sample after the rapid torque rise has taken place (Fig. 1). In order for the rapid increase in torque to be solely due to the amount of water present in the sample, then the 20% item would have to lose sufficient water (~3.4 g) to be at 12.5% water content. To test this possibility, two experiments were performed where the zein sample had 20% additional water. In the first experiment, the blend was sheared with a wall temperature of 90 °C for a total of two and one half minutes. In the second, the blend was sheared with a wall temperature of 90 °C for six and one half minutes. These points in time were selected as being immediately before and after the rapid increase in torque. After the desired time had been reached, the tests were stopped and as much of the sample as possible was removed from the chamber and its mass was determined (Table 3). As can be seen, the amount of material collected is very similar; suggesting that the amount of water lost between these two times is minimal.

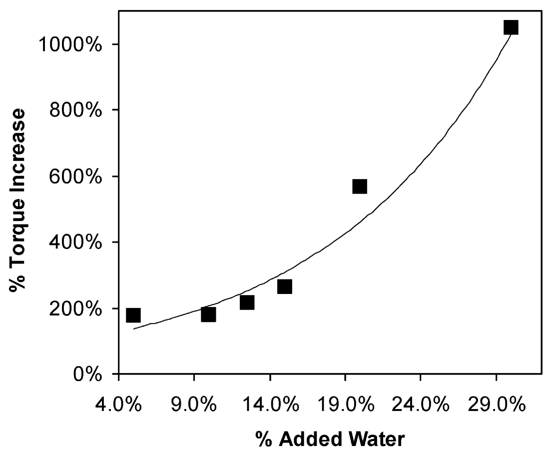

Fig. 3. Percent increase in torque versus % added water 10 min into trial. Equation for exponential line fitted to data, $y = 0.9224\ e^{8.0465}$ $R^2 = 0.936$.

Table 3
Mass of material collected at various times (50 rpm, 90 °C, roller) for zein with 20% additional water

Time (min)	Grams zein	Grams water	Starting total	Grams recovered
2.5	46.6	9.3	56	54.2
6.5	46.6	9.3	56	54.1

This rapid rise in torque, and viscosity, is not due to loss of water. Torque increases observed at later times during the run would be influenced by water loss.

It is has been suggested that these types of rapid increases in torque, are due to cross-linking, perhaps involving thiol groups [17]. The major zein fraction in Freeman zein is α zein and it has been shown to have only one thiol moiety per molecule (the number of thiol moieties per protein is dependent on which type of zein is being discussed, α, β, γ, or δ) [24,26,27]. There are others types of zein present in the material, which have more thiol moieties and these may allow disulfide chain extension, branching or cross-linking reactions to take place. In addition there are many hydroxyl and amide/acid moieties present in zein, and cross-linking reactions that would result in ester formation could possibly take place. Samples of zein that had undergone multiple torque transitions (20% water sample) as well as control zein were placed in glacial acetic acid (~6% solids). Both the treated material and control dissolved completely in a similar amount of time. This would suggest that cross-linking or other significant increases in molecular weight did not take place and that the torque increase is either due to protein aggregation or small amounts of chain extension/branching. Efforts are continuing to better understand the source of these rapid torque increases.

3.2. TEG

TEG is a known plasticizer for zein [23]. To determine if an organic plasticizer would interact in a different fashion than water, a series of TEG experiments were run (Table 2). The torque traces with time for most of these items were similar to the 10% water item, where there was observed a predominantly linear increase in torque with time (Fig. 4). Rapid torque increases were not observed in any of these experiments. The relationship between initial torque and percent TEG incorporated appears to be more linear rather than exponential as was seen with water (Fig. 2). This linear relationship between torque and %TEG is also observed 5, 10 and 20 min into the experiment. The differences between the two plasticizers may have to do with differences in rate of wetting, solvation, diffusion or dissolution [28]. Zein has regions that differ in polarity [25–27]. These differences may allow certain plasticizers to impact the rheology of the protein to a larger extent than others [25]. To determine if TEG can give the same rapid rise in torque seen with certain water levels, experiments were run at different temperatures to see if a rapid torque rise could be observed under different conditions. Initial wall temperatures were varied from 75 to 120 °C at a constant rpm of 50 on a blend of zein with 20% TEG. When the temperature reached 120 °C, the rapid rise in torque occurred seven minutes into the run. The magnitude of the increase is similar to that seen with 15% water. We believe that the same mechanism is responsible for the rapid viscosity rise displayed in both the TEG and water experiments. Detailed in Fig. 5 are the initial torque values for this temperature series. As expected, the initial torque needed to turn the rotors at 50 rpm decreases exponentially with increased temperature. An Arrhenius plot can be generated to obtain the energy of activation of flow by plotting the natural log of initial torque versus one over the initial temperature [29,30]. When this plot is produced the slope is found to be 5883.3 and the y-intercept is 13.383 with an R^2 of 0.9755. Assuming that torque and viscosity are linearly correlated for this series of data, the

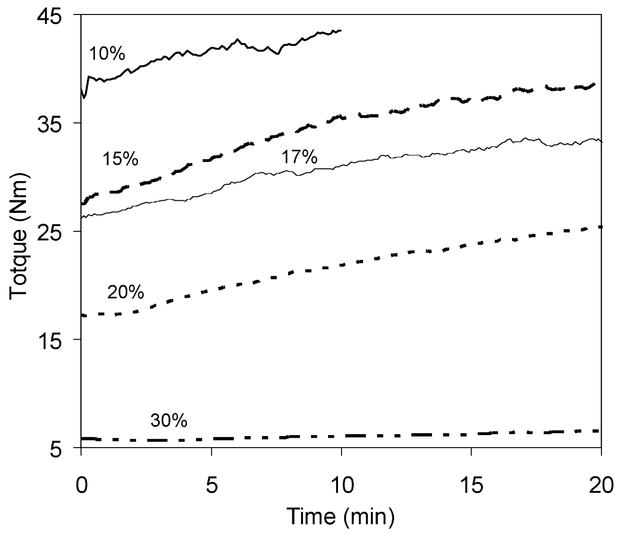

Fig. 4. Torque traces with time at various % TEG levels. Amount of TEG is expressed in % of zein (dry basis) and is detailed next to the respective trace.

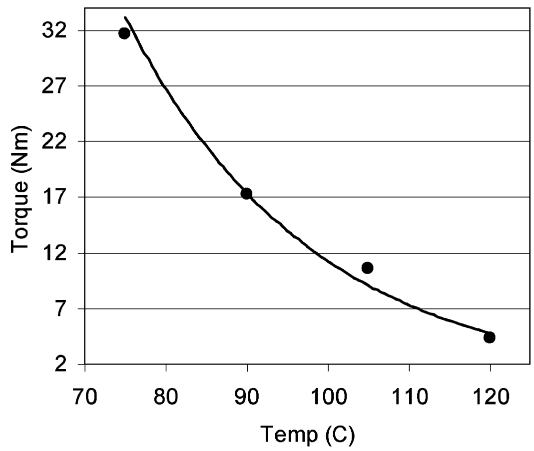

Fig. 5. Effect of wall temperature on initial torque for zein with 20% TEG. Equation for exponential line fitted to water data, $y = 847.9\ e^{-0.0432}$ $R^2 = 0.984$.

E_{act} flow is calculated to be 47.5 kJ/mol. This value is reasonable when compared to the values for low density polyethylene, high density polyethylene and nylon 1212 which are 37, 24 and 119 kJ/mol, respectively [30,31]. Knowing the E_{act} of flow is useful when developing commercial operations.

3.3. Water and TEG combinations

Experiments were run to determine what effect combinations of TEG and water had on the rate of torque rise (Table 2). If the %TEG is held constant at 17% and the %water is increased from 0 to 20%, the initial torque values decay exponentially with increased water (as was seen when water was used alone). The equation describing the relationship is detailed in Eq. (1). The R^2 for this data set was 0.95. When the torque traces of the zein, TEG and water combinations are examined over longer periods of time there is no clear trend in the rate at which the torque increases.

$$\text{Torque} = 17.701\ e^{-18.204(\%\text{water})} \quad (1)$$

The addition of 3.9% water to the 17% TEG provides a mixture that undergoes the rapid rise in torque that was observed when water in excess of 10% alone was used. Unlike those runs where water alone was used, the rapid rise occurred on the order of two more minutes later in the run than when water alone is used. These results would suggest that the TEG is affecting certain regions of the protein and after a certain degree of plasticization is achieved, the water is able to interact with the protein, allowing the rapid torque increase to take place. With TEG performing some degree of plasticization, a lower amount of water is needed to allow the rapid torque rise to take place. The resulting torque traces obtained from testing blends of zein and 17% TEG after adding 0, 3.3, 3.9, 7 or 20% water are displayed in Fig. 6. When 5 or 10% water was used without TEG, the rapid torque rise was not observed. When these lower levels of water were used in conjunction with 17% TEG, a rapid increase in torque was observed. For the combination of 17% TEG and 20% water, rapid torque increases are observed at two different times.

As observed with TEG alone, if the initial temperature of a TEG and water blend is increased, the rapid torque rise occurs where it was absent at a lower temperature. When 17% TEG is combined with either 2.3 or 3.3% additional water at 90 °C and 50 rpm, the rapid torque rise is not observed. When the temperature is increased to 105 °C, the rapid torque rise is observed in both of these formulations.

Rotor rpm was found to affect the time when the rapid torque rise took place. The impact on the time at which the torque increase takes place, is larger when the rotor rpm is reduced. When an experiment was run at 35 rpm for a 17% TEG and 15% water zein blend at 90 °C, the rapid rise took place 5.4 min into the run. At an rpm of 50, the rapid rise started approximately 3.2 min into the run. At an rpm of 65, the rapid rise started at a similar time, approximately 3.1 min into the run. This suggests that above a certain minimum value of shear, rapid torque increases will be relatively independent of shear. It is not clear if this difference is due to differences in mixing, allowing the plasticizer to work more efficiently, or if the shear aids in initiating those processes that increase the viscosity of the blend.

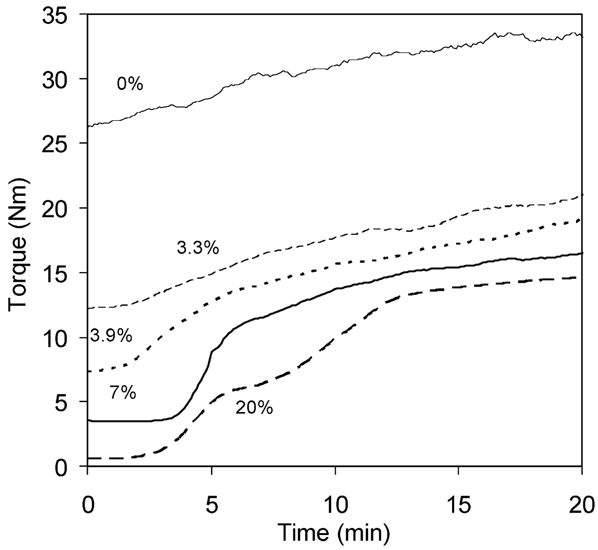

Fig. 6. Torque traces with time of zein, 17% TEG and various amounts of water. Amount of water is expressed in % of zein (dry basis) and is detailed next to the respective trace.

3.4. Water and TEG comparison

On a weight basis, water is a superior plasticizer when compared to TEG, however on a molar basis TEG is the superior plasticizer. In experiments using a single plasticizer, when the amount, in mmol, of either TEG or water is plotted versus initial torque, it is clearly seen that TEG

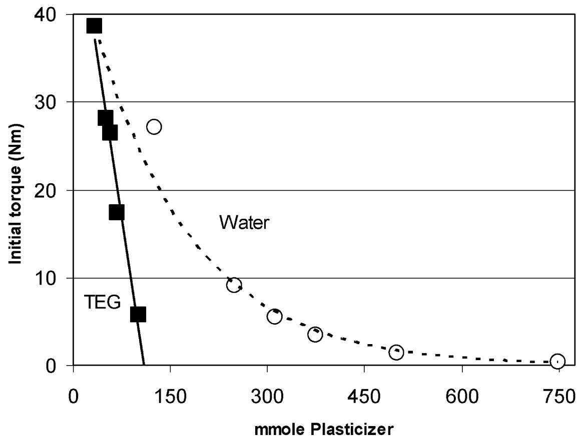

Fig. 7. Initial torque versus mmol water or mmol TEG. Equation for linear line fitted to TEG data, $y = -0.4879x + 53.37$ $R^2 = 0.973$. Equation for exponential line fitted to water data, $y = 48.26\ e^{-0.0066}$ $R^2 = 0.983$.

reduces torque more quickly than water (Fig. 7). Given that zein is more hydrophobic than most proteins, it is not surprising that water is a less effective plasticizer than TEG. In experiments conducted using corn gluten meal, water was also found to not be as effective a plasticizer as organic compounds capable of interacting with both hydrophilic and hydrophobic regions of the protein [25].

3.5. Model

A partially replicated, unbalanced completely randomized design (CRD) was used to examine the effects of varying levels of four experimental factors on torque. The four factors used in the study were % TEG, % water, temperature, and rpm. Batch size could not be used as a factor in the model, because experiments utilizing water could not be run at the higher batch size. By the time all of the sample could be added, torque had already begun to increase. A stepwise regression analysis was performed to obtain the best-fit equation for the dependent variable torque as a function of % TEG, % water, temperature, and rpm. All four independent variable factors were included in the analysis as possible main effects, as well as the six possible 2-factor interaction terms, the four possible 3-factor independent variable interaction terms and the 4-factor interaction term of all four variables. 95% confidence intervals on mean predicted values from the resulting regression equation were calculated for comparisons of torque at differing levels of the independent variables. The resulting best-fit equation obtained from stepwise linear regression of the results generated in this work is detailed in Eq. (2). For the equation having the best fit,

$$Y = 93.172 - 0.517X_4 - 79.878(X_2)^{0.385} - 1.523X_1X_3 - 0.558X_1X_4 - 149.521X_1X_2X_3 + 1.791X_1X_2X_3X_4 \quad (2)$$

where Y = torque, X_1 = %TEG, X_2 = %Water, X_3 = rpm, and X_4 = temperature.

only water and initial temperature impact torque directly, the other terms are secondary interactions. Measured torque results and predicted torque results that are generated from the model are detailed in Fig. 8. The R^2 of the line is 0.92. Being able to develop a model to a zein process in a torque rheometer gives confidence to being able to build a similar model to a zein process run in an extruder.

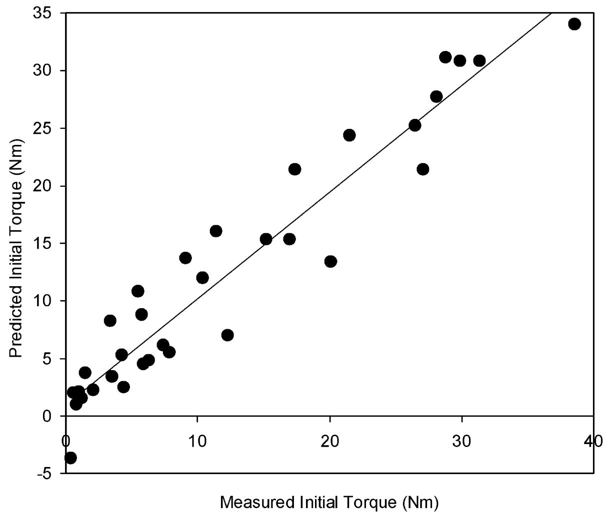

Fig. 8. Measured torque values versus predicted values based on model. Equation for linear line fitted to data, $y = 0.9248x + 0.9582$ $R^2 = 0.924$.

4. Conclusions

Water and TEG have been shown to interact differently with zein. With increasing amounts of water, the initial torque is reduced exponentially. With increasing amounts of TEG, the initial torque is reduced linearly. TEG is a more effective plasticizer when compared to water on a molar basis. Both TEG and water can induce rapid torque increases, however, TEG requires higher temperatures. When using TEG at these higher temperatures, the rapid torque increase takes longer to occur. At high levels of water or combinations of TEG and water, multiple morphological changes take place when sheared for sufficient time. Solubility of the zein isolated after undergoing these transitions suggests that cross-linking is not taking place. Information gathered to date cannot definitively determine if aggregation or dimerization/small degree of chain extension is occurring. For zein and TEG mixtures, the energy of activation for flow was found to be 47.5 kJ/mol. A model was developed allowing prediction of initial torque when using TEG and water under various conditions. The ability to accurately predict torque in a commercial mixer suggests that a similar model may be developed for zein, TEG and water (or other plasticizers) blends in an extruder. This ability to predict behavior demonstrates that the proteins that make up zein behave in a fashion similar to petroleum based synthetic polymers. These results suggest that additional studies carried out on a larger scale, using single or twin screw extruder, may be successful.

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